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Research paper

Influence of glucocorticosteroids on the biomechanical properties of *in-vivo* rabbit cornea

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ABSTRACT

Understanding corneal biomechanical responses during long-term glucocorticosteroids administration is important in clinical practice. The purpose of this study is to investigate the biomechanical influence of fluorometholone 0.1% eye drops on rabbit cornea. Thirty-eight Japanese white rabbits were randomly divided into three groups; a fluorometholone group, a supernatant group and a blank control group. For each rabbit in fluorometholone group, one cornea was treated with fluorometholone 0.1% eye drops four times a day for 8 weeks, while corneas of rabbits in supernatant group were treated in the same frequency with supernatant fraction centrifuged from fluorometholone 0.1% eye drops. The rabbits in the blank control group were not given any treatment. At the end of the 8 week observation period, the rabbits were euthanized and the eyes immediately enucleated and prepared for inflation testing. The experimental pressure-deformation data was used to derive the stress–strain behavior of each eye using an inverse modeling procedure. Comparisons of mechanical stiffness of corneas were conducted among the three groups to determine the influence of fluorometholone. The results showed that corneal stiffness decreased as the fluorometholone administration time prolonged. Comparisons of tangent modulus indicated average stiffness reductions of 34.2% and 33.5% in the fluorometholone group compared to the supernatant and control groups, respectively, at the end of the observation period. The stiffness-reduction effect of fluorometholone on the cornea should be considered in clinical management, especially when administrating it to biomechanically weakened corneas, such as after refractive surgeries and in cases of keratoconus.

Abbreviations: GCS, glucocorticosteroids; IOP, intraocular pressure; FM, fluorometholone; SN, supernatant; BC, blank control; RMS, root mean square; CCT, central corneal thickness; PCT, peripheral corneal thickness; E_t, tangent modulus

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1. Introduction

The cornea is a load-bearing tissue whose primary function is to focus light on the retina and protect the eye's internal components. The mechanical properties of the cornea are important in maintaining this function under the effect of actions such as intraocular pressure (IOP), eyelid movement and external impacts. Significant progress has been achieved using *in-vitro* tests to characterize the biomechanical properties of the cornea and to determine the effect of several influencing factors such as aging (Elsheikh et al., 2007), smoking (Spoerl et al., 2008) and the administration of glucose (Ni et al., 2011), glutaraldehyde (Spoerl and Seiler, 1999) and estrogen (Spoerl et al., 2007). Quantifying the changes in properties, and in particular mechanical stiffness, is important when these properties impact on the treatment of diseases such as keratoconus, accuracy of IOP measurements, outcome of refractive surgery procedures and design of contact lenses.

Glucocorticosteroid is a 21-carbon corticosteroid hormone produced by adrenal cortex. Due to their potent anti-inflammatory and immunosuppression actions, glucocorticosteroids (GCS) are commonly administered either locally or systemically in the treatment of ocular pathological conditions such as allergic conjunctivitis, uveitis, optic neuritis, Graves' ophthalmopathy, refractive surgery and as an anti-rejection agent after keratoplasty. However, GCS may cause adverse effects such as increases in the susceptibility to infections, diabetes, iatrogenic Cushing syndrome, cataract and glaucoma. They may also affect corneal stiffness similar to what has been found in tendons (Haraldsson et al., 2009), cartilage (Wang et al., 2005), bone (Liu et al., 2012), lungs (Choe et al., 2003), blood vessels (Reilly et al., 1990) and skin (Huscher et al., 2009), and in reducing the corneal wound strength in both rabbits and humans (Sugar and Chandler, 1974). The effect on corneal stiffness was evidenced in recent studies where incubation in a culture medium of 2.5 μM of hydrocortisone for 7 days markedly reduced the biomechanical stiffness of isolated cornea (Spoerl et al., 2009). However, the corneal strips used in the *in-vitro* study (Spoerl et al., 2009) were swollen and possibly unrepresentative of the normal physiological state. Further, the study did not simulate usual clinical administration methods such as topical eye drops or peribulbar injection, making the clinical significance of the results difficult to interpret. This paper attempts to quantify the effect of GCS on the mechanical stiffness of the cornea within a clinically relevant study where a form of GCS (fluorometholone) is administered as eye drops to rabbit corneas *in-vivo* and over a clinically-common administration period.

2. Materials and methods

2.1. Experimental animals

Thirty-eight Japanese white rabbits (2.0–3.0 kg) aged 3 months were obtained from the Animal Breeding Unit of Wenzhou Medical College. All animals were treated in agreement with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Care and Ethics Committee of Wenzhou Medical College.

2.2. Experimental design

The rabbits were randomly divided into three groups; 18 rabbits in a fluorometholone (FM) group, 10 rabbits in a supernatant (SN) group and 10 rabbits in a blank control (BC) group. In the FM group, one cornea was randomly selected and treated with fluorometholone 0.1% eye drops (5 mg/5 ml, Santen, Japan) four times a day for 8 weeks. In the SN group, one randomly-selected cornea was treated with a supernatant fraction centrifuged from fluorometholone 0.1% eye drops with the same frequency, while rabbits in the BC group were not given any treatment. Supernatant fraction was centrifuged from fluorometholone 0.1% eye drops by Centrifuge 5415D (eppendorf, Germany) with the rotation rate of 12,000 rcf. 0.1% fluorometholone eye drops belongs to aqueous suspensions, which contain fluorometholone particles and solvent. The solvent used as an additive contains some preservatives that include sodium edetate, benzalkonium fluoride ammonium and polysorbate 80. During the observation period of 8 weeks, IOP of each rabbit was measured once a week at approximately the same time of 8:00 am using a Tono-pen tonometer (Reichert, Inc., New York, USA). Each eye was measured three times, and the mean data were recorded to exclude rabbits with IOP over 21 mmHg since the long-term exposure to high IOP could potentially influence the corneal biomechanical properties (Sun et al., 2009).

2.3. Specimen preparation

At the end of the observation period, the rabbits were executed by intravenous injection of high concentrations of pentobarbital sodium (Merck, Darmstadt, Germany), and the treated eyes were immediately enucleated. The central and peripheral corneal thickness (approximately 1.5 mm away from the limbus) was then measured with an SP-3000 ultrasound pachymeter (Tomey, Japan) and corneal diameter measured using a vernier caliper in four directions. Each measurement was taken three times, and the mean data was recorded. The cornea and a 4 mm-wide ring of scleral tissue were mechanically separated from the eye globe using a pair of curved scissors, followed by the removal of the iris, lens and ciliary body. The specimens were then kept in a storage medium of Phosphate Buffered Saline (PBS-0060, Maixin, China) for a maximum period below 30 min until tested on an inflation rig.

2.4. Instrumentation and inflation testing procedures

As described previously (Elsheikh et al., 2008; Elsheikh and Anderson, 2005), a custom-made mechanical inflation test rig had been built to enable subjecting corneas to posterior pressure simulating the intraocular pressure. The trephinate specimen was mounted onto a specially designed pressure chamber of the rig using mechanical clamps and cyano-acrylate glue to provide

a watertight connection along the specimens' ring of scleral tissue (Fig. 1). The pressure chamber was filled with PBS and connected to a small reservoir, whose vertical movement was controlled by a step-motor (T21NRLC-LNN-NS-00, Rockford. IL, USA) to provide a tight control of posterior pressure. The pipeline that connected the pressure chamber to the reservoir passed through a water tank equipped with a temperature controller (Julabo, EHSY, China) to ensure the temperature of PBS in the pressure chamber remained at 37 °C throughout the test. The actual pressure in the chamber was measured using a pressure transducer (SK Ysen, Beijing, China) and the measurements were logged automatically.

The motor attached to the reservoir was controlled by Motion Assistant software (National Instruments Corporation, Texas, US) to set the pressure change rate at 25 mmHg/min. All specimens were subjected to a gradually increasing posterior pressure up to a maximum pressure of approximately 35 mmHg, which was above the normal physiological IOP level. Three cycles of loading and unloading up to 35 mmHg were applied to condition the tissue and stabilize its behavior before considering the results in the fourth cycle as representative of the cornea's biomechanical behavior. A recovery period of 90 s was allowed between each two conditioning cycles to enable specimens to recover their initial topography and to ensure the behavior was not affected by the strain history of preconditioning cycles (Carew et al., 2000).

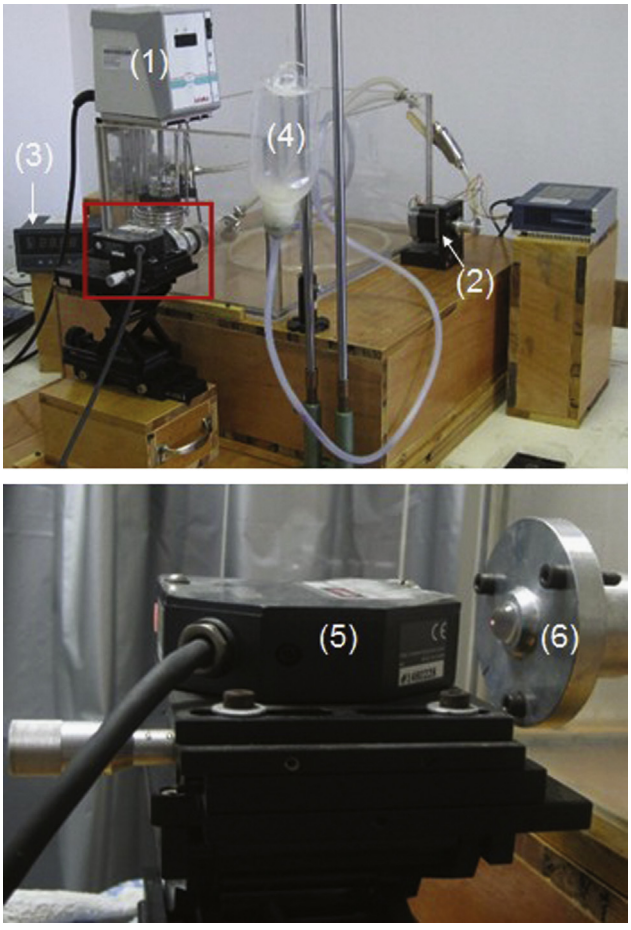

Fig. 1 – Overall view of inflation test rig showing temperature controller (1), step motor (2), pressure transducer (3), loading reservoir (4), laser displacement sensor (5), and pressure chamber (6).

A CCD laser displacement sensor (Keyence, Milton Keynes, UK) was used to continually monitor the displacement at corneal apex. The laser beam and the pressure transducer were connected to a personal computer to record the data automatically for later analysis. Before the fourth loading cycle, the laser sensor was moved vertically and horizontally across the width of the specimen to determine corneal outer profile in the superior–inferior and temporal-nasal directions.

2.5. *Inverse modeling*

An inverse modeling procedure was used to characterize the mechanical properties of the rabbit corneas based on the inflation pressure-deformation results. The method used the optimization software HEEDS 6.1 (Red Cedar Technology, Inc., East Lansing, MI, USA) coupled with the nonlinear finite element software ABAQUS 6.10 (Dassault Systèmes Simulia Corp. Rhode Island, USA). HEEDS utilized the SHERPA algorithm which uses a combination of local and global search methods to find the optimal material parameters (Bischoff et al., 2009). To model the corneal mechanical behavior, the first-order, hyperelastic Ogden material model was used

$$W = \frac{2\mu}{\alpha^2}(\bar{\lambda}_1^{\alpha} + \bar{\lambda}_2^{\alpha} + \bar{\lambda}_3^{\alpha} - 3) + \frac{1}{D}(J-1)^2 \quad (1)$$

where $\bar{\lambda}_1$, $\bar{\lambda}_2$, $\bar{\lambda}_3$ represent the deviatoric principal stretches $= J^{-1/3}\lambda_k$ ($k=1, 2, 3$), λ_1, λ_2, λ_3 are the principal stretches, $J=\lambda_1\lambda_2\lambda_3$, and α, μ and D are the material constants. D is a compressibility parameter, whose value is dependent on that of Poisson's ratio, υ, in the form $=(3(1-2\upsilon))/(\mu(1+\upsilon))$. With reported values of υ for corneal tissue between 0.46 and 0.5 (Battaglioli and Kamm, 1984), a value of 0.48 was assumed in this study, making $D=0.081/\mu$. On the other hand, μ and α were obtained from the inverse modeling procedure. Their values were allowed to vary between 0.01 and 0.2, and between 50 and 300, respectively, which were suitable for all specimens included in the study. Parameter uniqueness was tested in three randomly selected specimens (one in each group) by repeating the inverse analysis two more times with wider μ and α ranges and while using initial values that were set at double and half the final values of the first analysis.

The objective function of the optimization process was the root mean square (RMS) of the error between the measured and computed apex displacements at the same level of IOP

$$RMS = \frac{1}{N}\sqrt{\sum_{i=1}^{N}(U_i^{exp} - U_i^{num})^2} \quad (2)$$

where N is the number of points along the pressure–displacement relationship considered in the inverse modeling, U^{exp} and U^{num} are the measured and the computed apex longitudinal displacements, respectively. A total of 300 iterations were found to be sufficient to find the optimum values for all cases with stable values of RMS error.

A 3D finite element model of each cornea was created using a custom-written Visual Basic program utilizing the measured values of corneal diameter, thickness at the limbus and apex, and profile in the inferior–superior and temporal-nasal

directions. Each model consisted of 44,205 nodes and 13,824, 15-noded, continuum elements arranged in 4 layers and 24 circular rings as illustrated in Fig. 2. The IOP was applied as a uniform pressure to the elements' internal surface. Nodes on the corneoscleral junction were assumed pinned to simulate the mechanical clamping of the inflation rig.

2.6. Statistical analysis

All analyses were performed using PASW Statistics software v18.0 (IBM Corporation, USA). Continuous variables were expressed as mean ± standard deviation (SD). Comparisons of data such as corneal thickness and diameter among the three specimen groups were performed using one-way ANOVA. Comparisons of tangent modulus were performed using the Kruskal–Wallis H test, and the multiple comparisons were made using the Nemenyi test. In this study, P-values of less than 0.05 were considered indicative of statistical significance.

3. Results

3.1. Corneal thickness, diameter and IOP values

Apart from three rabbits in the fluorometholone group, in which IOP was higher than 21 mmHg, IOP values ranged from 11 to 18 mmHg. Three rabbits died in the fluorometholone group during the observation period probably due to pneumonia. This left 12, 10 and 10 rabbits in the three groups.

The average central corneal thickness (CCT) and the corresponding peripheral corneal thickness (PCT) in the three groups are given in Table 1. CCT was larger than PCT in all corneas, and the average difference was 17.7 ± 4 μm (4.7 ± 1%), 19.5 ± 5.6 μm (4.6 ± 2.1%) and 24.7 ± 7.4 μm (6.4 ± 1.9%) for specimens in fluorometholone, supernatant and blank control group, respectively. The differences in corneal thickness between the three groups were not statistically significant (P = 0.221), and a similar observation was made for corneal diameter measurements (P = 0.236).

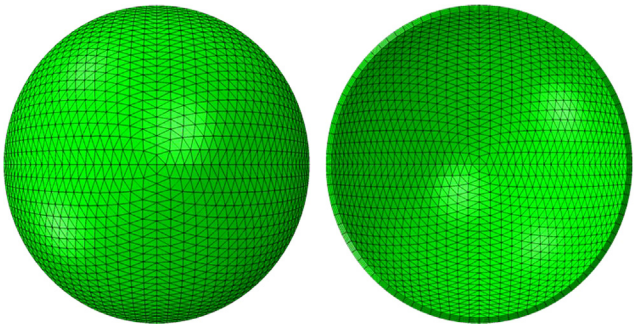

Fig. 2 – (a) Anterior and (b) posterior views of a numerical model.

3.2. Experimental behavior

The pressure–apical rise behavior of all specimens in the three groups is compared in Fig. 3. In all cases, specimens exhibited an initial low stiffness and a final, considerably higher, stiffness (as measured by the tangent to the pressure–displacement curve). Comparing the pressure–displacement relationships for the three groups in Fig. 3D show a clear difference in behavior with specimens in the fluorometholone group exhibiting lower stiffness up to an applied pressure of approximately 15 mmHg, beyond which all specimens followed a linear behavior with almost the same stiffness.

3.3. Material constitutive models

Inverse modeling was used to estimate the material parameters (μ, α) that provided the best fit with the experimental results for each specimen, Table 2. The average, standard deviation and range of RMS errors for the fluorometholone, supernatant and blank control groups were 0.016 ± 0.004 mm (range: 0.010–0.024 mm), 0.010 ± 0.002 mm (range: 0.005–0.013 mm) and 0.010 ± 0.005 mm (range: 0.005–0.019 mm), respectively. The uniqueness tests were conducted for three specimens, whereby inverse modeling analyses were repeated twice with the initial values of μ and α set at half and twice their final values of the first analysis, and produced the same μ and α parameters. Fig. 4 shows example comparisons between the pressure–apical rise behavior as recorded experimentally and predicted numerically for three typical specimens from the three groups. Further, Fig. 5 presents the stress–strain behavior patterns for all corneas as obtained from the inverse modeling exercise, along with a comparison between the average behavior of the three groups. The comparisons demonstrate clearly lower stiffness of corneas in the fluorometholone group.

The stress–strain constitutive models also enabled the determination of the tangent modulus (E_t, a measure of material stiffness) at different stress levels: $E_t = d\sigma/d\varepsilon \approx \Delta\sigma/\Delta\varepsilon$. In this study, the E_t–σ relationships were assessed for all specimens and the results demonstrated that specimens in the fluorometholone group were significantly softer than the other two groups. In order to quantify this effect, the tangent modulus was calculated for each specimen at three stress levels (0.001, 0.002 and

Table 1 – Average and standard deviation values of corneal thickness and diameter within each group. Statistical analyses were performed using one-way ANOVA.

Group	n	CCT (μm)	PCT (μm)	Diameter (mm)
Fluorometholone group	12	370 ± 14	352 ± 12	13.83 ± 0.15
Supernatant group	10	377 ± 19	359 ± 14	13.74 ± 0.22
Blank control group	10	383 ± 10	358 ± 9	13.71 ± 0.11
P-value		0.141	0.324	0.236

CCT = central corneal thickness and PCT = peripheral corneal thickness.

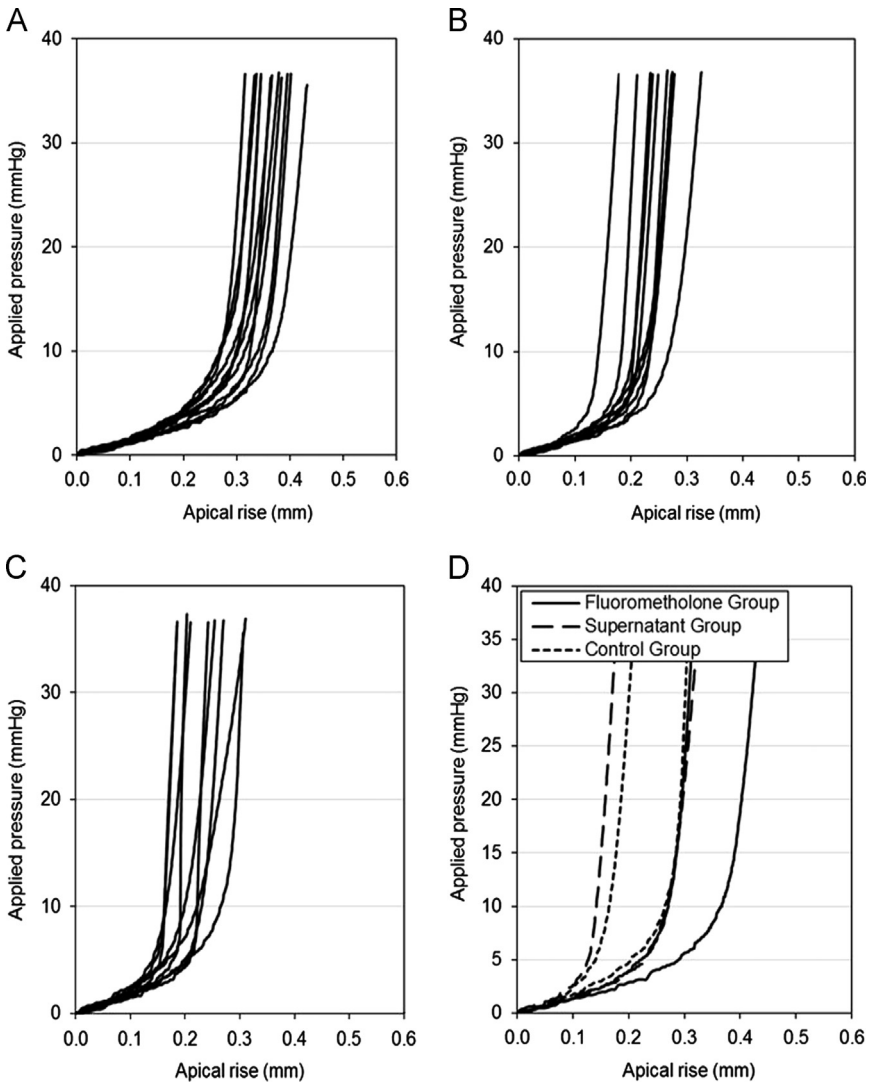

Fig. 3 – Experimental pressure–apical rise behavior obtained for specimens in (A) fluorometholone group, (B) supernatant group and (C) blank control group. (D) A comparison between the behavior ranges for the three specimen groups.

0.004 MPa), which were equivalent to pressures of approximately 7.5, 15 and 30 mmHg, respectively. The average and standard deviation values for each specimen group are presented in Table 3 and show consistent increases in E_t associated with higher stress as would be expected from a hyperelastic material such as the cornea. The Kruskal–Wallis H test was used to compare the modulus values in the three groups and all *P*-values were below 0.01. Multiple comparisons showed that the modulus values in the fluorometholone group were significantly lower than those in the supernatant and blank control group, but the modulus values in the supernatant group were not significantly different from the blank control group. On average, E_t in the fluorometholone group was lower by 34.2% and 33.5% compared to the supernatant group and the blank control group, respectively.

4. Discussion

The common use of glucocorticosteroids (GCS) in clinical practice and the possible effect they have on the mechanical properties of various tissues make it important to characterize their stiffness-reducing effect on the cornea especially in cases where it has been weakened in refractive surgeries or advanced keratoconus. Only one study published on this topic, while finding correlation between GCS and stiffness reduction, did not simulate usual clinical administration methods such as topical eye drops and peribulbar injection.

The main finding of the present study was that corneal tissue had significantly lower material stiffness (tangent modulus) after 8 weeks of treatment with fluorometholone 0.1% eye drops compared to eyes treated with a supernatant fraction and untreated control eyes. The results demonstrating a significant effect of fluorometholone on corneal mechanical properties using an *in-vivo* model are compatible with previous data obtained using an *in-vitro* model of incubation in culture medium of 2.5 μM of hydrocortisone for 7 days (Spoerl et al., 2009). The difference in stiffness reduction between the two studies (42.8% in *in-vitro* study versus 33.5% in the present *in-vivo* data) could be due to the use of different GCS concentrations and administration methods, different test periods and the possible variation in tissue behavior between *in-vivo* and *in-vitro* states.

Table 2 – Ogden material parameters of all specimens and the RMS of error of fit with the experimental results.

Rabbit	Fluorometholone group			Rabbit	Supernatant group			Rabbit	Control group		
	μ	α	RMS		μ	α	RMS		μ	α	RMS
1	0.050	118.8	0.021	13	0.070	134.0	0.011	23	0.190	115.0	0.005
2	0.100	099.8	0.011	14	0.090	156.8	0.008	24	0.190	145.4	0.006
3	0.080	111.2	0.017	15	0.070	156.8	0.011	25	0.070	164.4	0.011
4	0.103	092.2	0.015	16	0.100	153.0	0.008	26	0.130	153.0	0.005
5	0.110	096.0	0.011	17	0.050	179.6	0.013	27	0.060	225.2	0.016
6	0.110	107.4	0.010	18	0.080	187.2	0.011	28	0.050	194.8	0.016
7	0.050	107.4	0.020	19	0.050	194.8	0.010	29	0.100	210.0	0.003
8	0.060	097.0	0.024	20	0.050	200.0	0.010	30	0.130	127.4	0.019
9	0.060	118.8	0.016	21	0.060	225.2	0.009	31	0.060	259.4	0.009
10	0.070	130.2	0.014	22	0.110	263.2	0.005	32	0.060	267.0	0.009
11	0.100	111.2	0.012								
12	0.060	103.6	0.016								
Average	0.079	107.8	0.016		0.073	185.0	0.010		0.104	186.1	0.010
SD	0.023	011.1	0.004		0.021	038.4	0.002		0.053	053.5	0.005

SD=standard deviation and RMS=root mean square.

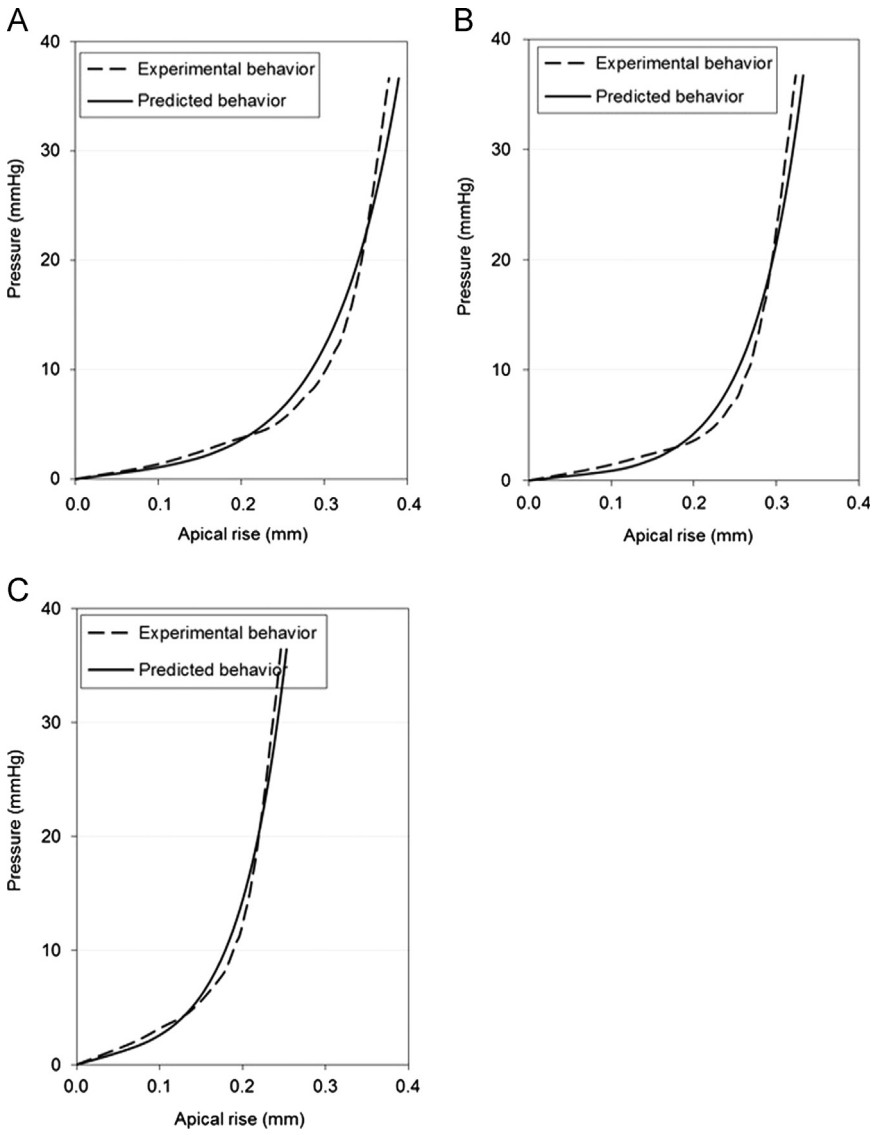

Fig. 4 – Comparisons between the pressure–displacement behavior as obtained experimentally and predicted numerically for a typical specimen from each of (A) the fluorometholone group, (B) the supernatant group and (C) the blank control group.

The mechanical performance of the cornea is dominated by the stroma, which is composed of an extracellular matrix mainly formed of type I fibrillar collagens and proteoglycans. Since type I collagens are synthesized by keratocytes, alterations in keratocytes' function could affect corneal mechanical performance. In previous studies, glucocorticosteroids, such as dexamethasone, have been shown *in-vitro* to inhibit keratocyte proliferation and physiological function under particular conditions (Bourcier et al., 1999; Lu et al., 1996). Dexamethasone-induced keratocyte apoptosis and necrosis led to less production of type I fibrillar collagens and other components of the extracellular matrix, which could affect the structural integrity of the cornea and weaken it mechanically. Moreover, other studies found therapeutic doses of corticosteroids significantly decrease the type I collagen messenger RNA (mRNA) expression and inhibit the proliferation and activity of tendon tenocytes (Tsai et al., 2002; Wong et al., 2004), which lead to suppression in collagen I production (Chen et al., 2007). These corticosteroid-associated disturbances of tendon cell metabolism may affect the structural integrity of the tendon and hence its mechanical properties (Haraldsson et al., 2009), and a similar effect on corneal mechanical performance could further be expected due to the administration of corticosteroid and the subsequent suppression in collagen synthesis.

Furthermore, corticosteroids have been found to increase the production and activity of collagenolytic enzymes, such as matrix metalloproteinases (MMPs)-2 and MMP-9 (Hillegass et al., 2008; Lee et al., 2003), which are lytic enzymes that can degrade proteins such as collagen (Brown et al., 1970). Therefore, the corticosteroids-induced excessive production of MMPs in the cornea can result in an accelerated degradation of collagen fibers (types I, III, V and VII) and extracellular matrix components (elastin, fibronectin, laminin and proteoglycans) (Birkedal-Hansen et al., 1993; Cury et al., 2007), and ultimately lead to deterioration in corneal stiffness.

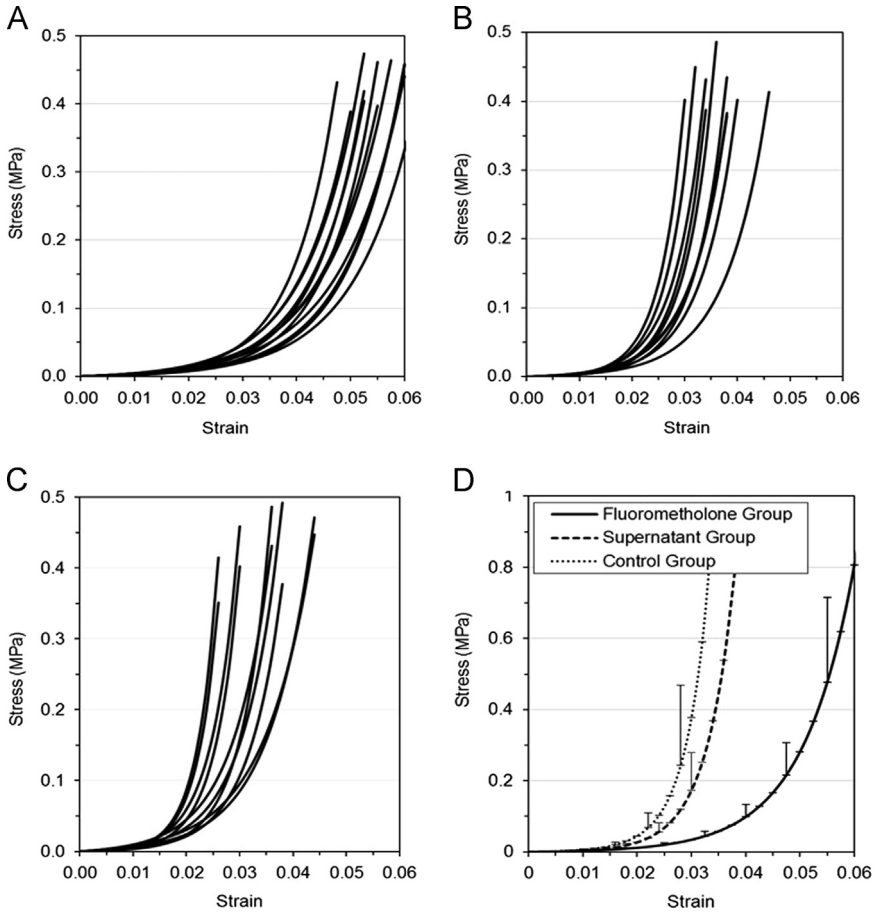

Fig. 5 – Stress–strain behavior of corneal tissue based on the material parameters obtained from the inverse modeling study for specimens in (A) fluorometholone group, (B) supernatant group and (C) blank control group. (D) A comparison between the average stress–strain behavior for the three specimen groups.

Table 3 – Tangent modulus values that correspond to specific levels of corneal stress for each specimen group.

Group	*n*	Tangent modulus (MPa) at stress		
		0.001 MPa	0.002 MPa	0.004 MPa
Fluorometholone group	12	0.138±0.015	0.230±0.024	0.412±0.048
Supernatant group	10	0.190±0.049	0.355±0.069	0.687±0.157
Blank control group	10	0.196±0.063	0.348±0.123	0.653±0.243

One possible effect of the decrease in corneal mechanical stiffness caused by corticosteroids is on keratoconus progression. Keratoconus is a noninflammatory corneal disease characterized by stiffness reduction leading to progressive thinning of the central cornea and keratectasia (Krachmer et al., 1984; Rabinowitz, 1998). A recently developed therapy of keratoconus involves the use of photosensitizer riboflavin and ultraviolet A radiation to cross-link the collagen fibers, increase corneal stiffness and hence arrest keratoconus progression. However, previous studies found that corticosteroids administered to keratoconic eyes after collagen cross-linking (CXL) may cause resumption of keratoconus progression and need for repeat cross-linking procedures (Raiskup-Wolf et al., 2008; Spoerl et al., 2009). This finding is compatible with our experimental results confirming the effect of topical corticosteroids eye drops on the mechanical stiffness of the cornea.

Another possible effect of the stiffness reduction could be associated with the routine use of topical glucocorticosteroids to prevent postoperative inflammatory and immune responses after refractive surgeries (Vetrugno et al., 2001, 2000). The development of keratectasia especially in cases with thin residual stromal bed, preoperative forme fruste keratoconus, pellucid marginal degeneration and higher amplitudes of refractive correction could be partly due to the effect of GCS in reducing the stiffness of the residual cornea and hence encouraging deformation under IOP.

The corneal stiffness reduction has another possible effect in underestimating the value of IOP using tonometry techniques

(Elsheikh et al., 2006). With the stiffness losing as much as 34.2% of its pre-treatment value, the effect on IOP measurement could lead to cases of false negatives and affect the management of glaucoma patients as has been found in an earlier study (Worley and Grimmer-Somers, 2011).

The stiffness-reducing effect of GCS is not limited to the cornea, and has been reported in other tissues including rat-tail tendons (Haraldsson et al., 2009) and bones (Liu et al., 2012) (following daily administration for 4 weeks). Other examples include the lungs where systemic treatment with 2 mg/kg of methylprednisolone daily for 4 weeks upregulated the activity of MMPs and caused emphysema occurrence in rats (Choe et al., 2003). The role of GCS in the induction of aneurysms, aortic ectasia or aortic rupture in mice that received 0.45 mg/mL of hydrocortisone acetate in drinking water for 12 days was also established (Reilly et al., 1990). These studies point at possible serious side-effects of the long term exposure to GCS, which should be considered in clinical practice

There are a number of limitations related to this study. In addition to the fixed boundary conditions created by the clamps, the use of a single clamp size coupled with the inter-specimen variation in diameter (13.36–14.07 mm) would be expected to induce nonphysiological stresses in the adjacent tissue. However, the effect of these stresses was found to diminish by the limbus in a trial FE analysis. A more significant limitation is the assumption of material homogeneity in the inverse modeling procedure. This assumption was adopted as it was necessary to avoid the difficulties associated with behavior comparisons involving different material models in different parts of the cornea. Further, the study relied on rabbit corneas due to difficulties in obtaining human specimens. Since rabbit corneas are thinner than human corneas and rabbits have a much smaller body weight than humans, the amount of fluorometholone 0.1% eye drops used four times a day in human eyes may produce a lower effect on corneal stiffness than what has been found in the study on rabbit eyes. Additionally, the study concentrated on the changes in stiffness at the end of an 8 week observation period. No information could be gathered on the gradual reduction in stiffness over this period or on whether the cornea was likely to reverse the stiffness deterioration after the end of the fluorometholone administration period.

5. Conclusion

In conclusion, the present study demonstrated the effects of long-term glucocorticosteroids administration on the mechanical stiffness of rabbit cornea. Corneal stiffness was reduced by up to 33.5% compared with the untreated controls after an 8-week intervention period. This significant stiffness reduction could have important implications on clinical practice involving the local administration of GCS eye drops, especially in cases with increased risk of keratoconus progression and post-LASIK ectasia.

Acknowledgments

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